In re: Rao, et al. 'Appl. No.: 09/478,598 Filed: 1/6/2000

Page 2

56. The method of Claim 55, wherein said antibodies are monoclonal antibodies.

Jul G14 5 proteins.

57. The method of Claim 54 wherein said interacting molecules are dimerizing

58. A method for altering amino acid composition of a native protein of interest whose conformation is unavailable, said method comprising introducing amino acid changes into said protein to create an engineered protein, said engineered protein having the conformation of the native protein wherein said conformation of the engineered protein is confirmed by binding said engineered protein with a set of antibodies capable of binding with the native protein, wherein said antibodies recognize native conformation, and wherein said amino acid changes are made to increase levels of essential amino acids in the engineered protein.

59. The method of Claim 58, wherein said essential amino acids are selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, theronine and cysteine.

60. The method of Claim 58, wherein said amino acid changes [involve] comprise increasing the levels of methionine.

- 61. The method of Claim 54, wherein said amino acid changes are introduced into predetermined sites.
- 62. The method of Claim 61, wherein said predetermined sites are determined by secondary structure prediction or homology comparison.

pro

butla

In re: Rao, et al. 'Appl. No.: 09/478,598 Filed: 1/6/2000

Page 3

random.

63. The method of Claim 54, wherein said amino acid changes are introduced at

64. The method of Claim 62, wherein said amino acid changes are produced by mutagenic PCR, DNA shuffling, or phage display methodology.

65. The method of Claim 64, wherein correctly folded variants are confirmed by filter lift assay or ELISA.

66. The method of Claim 58, wherein said essential amino acids are increased to represent 5% of the total amino acid content of the protein.

- 67. The method of Claim 58, wherein said essential amino acids are increased to represent 10% of the total amino acid content of the protein.
 - 68. The method of Claim 54, wherein said protein is vegetative storage protein.
- 69. A method for altering amino acid composition of a native protein of interest, said method comprising introducing amino acid changes into said protein to create an engineered protein having increased nutritional value, said engineered protein having the conformation of the native protein wherein said conformation of the engineered protein is confirmed by binding said engineered protein with a set of interacting molecules capable of binding with the native protein, and wherein said molecules recognize native conformation.
 - 70. The method of Claim 69, wherein said interacting molecules are antibodies.
 - 71. The method of Claim 70, wherein said antibodies are monoclonal antibodies.

July 4

In re: Rao, et al.

Appl. No.: 09/478,598

Filed: 1/6/2000

Page 4

Sugar

72. The method of Claim 69 wherein said molecules are dimerizing proteins.

phos finds 73. The method of Claim 69, wherein said amino acid changes are made to increase levels of essential amino acids in the engineered protein.

- 74. The method of Claim 73, wherein said essential amino acid is selected from the group consisting of methionine tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, theronine and cysteine.
 - 75. The method of Claim 74, wherein said essential amino acid is methionine.
- 76. The method of Claim 69, wherein said amino acid changes are introduced into predetermined sites.
- 77. The method of Claim 76, wherein said predetermined sites are determined by secondary structure prediction or homology comparison.
- 78. The method of Claim 69, wherein said amino acid changes are introduced at random.
- 79. The method of Claim 69, wherein said amino acid changes are produced by mutagenic PCR, DNA shuffling, or phage display methodology.
- 80. The method of Claim 79, wherein correctly folded variants are confirmed by filter lift assay or ELISA.

In re: Rao, et al.
Appl. No.: 09/478,598
Filed: 1/6/2000

Page 5

pulcy

- 81. The method of Claim 73, wherein said essential amino acids are increased to represent 5% of the total amino acid content of the protein.
- 82. The method of Clarm 73, wherein said essential amino acids are increased to represent 10% of the total amino acid content of the protein.
 - 83. The method of Claim 69, wherein said protein is vegetative storage protein.
- 84. An engineered protein having altered amino acid composition, wherein said amino acid composition has been altered by introducing amino acid changes into said protein, wherein said engineered protein binds to a set of interacting molecules capable of binding with a corresponding native protein, and wherein said molecules recognize native conformation.
 - 85. The protein of Claim 84 wherein said interacting molecules are antibodies.
 - 86. The protein of Claim 85, wherein said antibodies are monoclonal antibodies.
- 87. The protein of Claim 84, wherein said amino acid changes increase the levels of essential amino acids in the protein.
- 88. The protein of Claim 87, wherein said essential amino acids are selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, theronine and cysteine.
 - 89. The protein of Claim 88, wherein said essential amino acids are increased to represent 5% of the total amino acid content of the protein.

In re: Rao, et al. Appl. No.: 09/478,598

Filed: 1/6/2000

Page 6

- 90. The protein of Claim 87, wherein said essential amino acids are increased to represent 10% of the total amino acid content of the protein.
- 91. The protein of claim 87, wherein said amino acid changes [involve increasing] increase the level of methionine.
- 92. The protein of Claim 84, wherein said amino acid changes are introduced into predetermined sites.
- 93. The protein of Claim 92, wherein said predetermined sites are determined by secondary structure prediction or homology comparison.
- 94. The protein of Claim 84, wherein said amino acid changes are introduced at random.
- 95. The protein of Claim 92, wherein said amino acid changes are produced by mutagenic PCR, DNA shuffling, or phage display methodology.
 - 96. The protein of Claim 84, wherein said protein is vegetative storage protein.

97. A method for altering amino acid composition of a vegetative storage protein, said method comprising introducing amino acid changes into said protein to create an engineered protein, said engineered protein having the conformation of a native vegetative storage protein wherein said conformation of the engineered protein is confirmed by binding with a panel of monoclonal antibodies capable of binding said native vegetative storage protein, and wherein said antibodies recognize native conformation.

In re: Rao, et al. . Appl. No.: 09/478,598 Filed: 1/6/2000

Page 7

98. The method of Claim 97, wherein said amino acid changes are made to increase levels of essential amino acids in the engineered protein.

99. The method of Claim 98, wherein said essential amino acids are selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, theronine and cysteine.

- 100. The method of Claim 98, wherein said amino acid changes comprise increasing the level of methionine.
- 101. The method of Claim 97, wherein said amino acid changes are introduced into predetermined sites.
- 102. The method of Claim 101, wherein said predetermined sites are determined by secondary structure prediction or homology comparison.
- 103. The method of Claim 97, wherein said amino acid changes are introduced at random.
- 104. The method of Claim 103, wherein said amino acid changes are produced by mutagenic PCR, DNA shuffling, or phage display methodology.

105. The method of Claim 104, wherein correctly folded variants are confirmed by filter lift assay or ELISA.

106. The method of Claim 98, wherein said essential amino acids are increased to represent 5% of the total amino acid content of the engineered protein.

W.

busha

In re: Rao, et al. Appl. No.: 09/478,598

Filed: 1/6/2000

Page 8

Ch's

107. The method of Claim 98, wherein said essential amino acids are increased to represent 10% of the total amino acid content of the engineered protein.

108. An engineered vegetative storage protein having altered amino acid composition, wherein said amino acid composition has been altered by introducing amino acid changes into said protein, wherein said protein binds to a set of monoclonal antibodies capable of binding with a corresponding native vegetative storage protein, and wherein said antibodies recognize native conformation.

put [73]

- 109. The protein of Claim 108, wherein said essential amino acids are selected from the group consisting of methionine, tryptophan, lysine, valine, phenylalanine, isoleucine, leucine, theronine and cysteine.
- 110. The protein of Claim\109, wherein said essential amino acids are increased to represent 5% of the total amino acid content of the protein.
- 111. The protein of Claim 109, wherein said essential amino acids are increased to represent 10% of the total amino acid content of the protein.
- 112. The protein of claim 84, wherein said protein is selected from the group consisting of:
 - a) a protein having the amino acid sequence set forth in SEQ ID NO: 8;
 - b) a protein having the amino acid sequence set forth in SEQ ID

 NO: 9; and
 - c) a protein having the amino acid sequence set forth in SEQ ID NO:

In re: Rao, et al.
Appl. No.: 09/478,598
Filed: 1/6/2000
Page 9

113. The protein of claim 84, wherein said protein has the amino acid sequence set forth in SEQ ID NO: 8.

114. The protein of claim 108, wherein said corresponding native vegetative storage protein is a *Glycine max* vegetative storage protein β.

- 115. A method for altering amino acid composition of a native protein of interest, said method comprising introducing amino acid changes into said protein to create an engineered protein having increased level of essential amino acids, said engineered protein having the conformation of the native protein wherein said conformation of the engineered protein is confirmed by binding said engineered protein with a set of interacting molecules capable of binding with the native protein, and wherein said molecules recognize native conformation.
 - 116. The method of claim 115, wherein said molecules are monoclonal antibodies.
- 117. A method for altering amino acid composition of a native protein of interest whose conformation is unavailable, said method comprising: introducing amino acid changes into said protein to create an engineered protein, said engineered protein having the conformation of the native protein wherein said conformation of the engineered protein is confirmed by binding said engineered protein with a set of interacting molecules capable of binding with the native protein, wherein said molecules recognize native conformation, and wherein sites of said changes are selected by at least two methods selected from methods consisting of:
 - a) mutational analysis
 - b) secondary structure prediction
 - c) homology comparison.

JUC.